a propagating wave of depolarization to adjacent myocytes. These insights can be used to better understand the basic mechanisms of calcium-entrained cardiac arrhythmias.

3025-Pos Board B130
Self-Organization of Pacemaking Sites for Calcium Waves and Oscillations in Cardiac Myocytes
Michael Nivala, Chris Ko, Alan Garfinkel, James N. Weiss, Zhilin Qu.
Calcium (Ca) sparks are elementary events of intracellular Ca signaling, which tend to occur randomly. Ca waves and whole-cell Ca oscillations occur under Ca overload and disease conditions. How Ca waves emerge from Ca sparks is not completely understood. We developed a three-dimensional model for Ca cycling which contains 100x20x10=20,000 identical Ca release units (CRUs), simulating the CRU network corresponding to a complete cardiac myocyte with dimensions of 100x20x10 micrometers. Using this model, we can generate the well known Ca signaling hierarchy: Ca quarks, Ca sparks, macro-sparks, abortive waves, and full Ca waves. We can also induce spiral waves within the cell, a wave phenomenon widely observed in myocyte experiments. Besides the well known experimental observation that increasing Ca loading promotes these wave dynamics, we also make the following observations: 1) The diffusion rate of Ca is a key parameter. Spontaneous Ca waves occur only when the diffusion rate is above a critical value. 2) When the model is homogeneous, Ca waves originate from different locations via a self-organizing process. This self-organizing process is influenced by, but does not require, heterogeneity. 3) When the model contains heterogeneities, such as heterogeneous Ca release channel distribution, Ca waves can originate from different locations or occur repeatedly from the same location. In real cardiac rabbit ventricular myocytes loaded with Fluo-4 AM to image intracellular Ca, Ca waves typically originate from different locations after successive rapid pacing episodes. In conclusion, our results indicate that Ca waves in cardiac myocytes originate predominantly as a result of self-organizing processes rather than pre-existing heterogeneities.

3026-Pos Board B131
Ca^{2+} Leak and Ca^{2+} Sparks in Mammalian Heart: Insights from a Computational Model
George S.B. Williams, Aristide C. Chikando, W. Jonathan Lederer, Eric A. Sobie, Hoang-Trong M. Tuan, M Saleet Jafri.
Calcium (Ca2+) signaling in muscle, neuronal, and non-excitable cells has benefited significantly from advances in biological tools and imaging technology, however, the molecular interactions of nanoscopic molecules, structures and compartments has been challenging to study under physiological conditions. Here, we exploit novel computational modeling techniques to examine real-time molecular and cellular physiology in cardiac ventricular myocytes. The model focuses on local and cell-wide Ca2+ signaling phenomena related to calcium induced calcium release from intracellular calcium channels, ryanodine receptors (RyR2s), located on the sarcoplasmic reticulum (SR) membrane. This work is informed by the latest molecular investigations and recent characterizations of channels, transporters, and buffers located in mammalian heart. We have created a detailed, whole-cell model of Ca2+ signaling using a realistic number of calcium release units (CRU) each containing a cluster of stochastically gating RyR2s. During systole the opening of these RyR2s is triggered by Ca2+ entry via voltage gated L-type Ca2+ channels. The synchronized opening of the RyR2 cluster leads to localized elevations of [Ca2+]i known as Ca2+ sparks. During diastole Ca2+ sparks are still observed and are attributed to the finite opening rate of the RyR2. RyR2s are also believed to display unsynchronized or non-spark openings where only a few channels in the CRU open without triggering the remainder of the RyR2 cluster. This non-spark Ca2+ release would be below current experimental detection thresholds and therefore ‘‘invisible.’’ These spark and non-spark openings of RyR2s constitute a molecular basis for Ca2+ leak from the SR. The computational model suggests that a significant fraction of SR Ca2+ leak is due to RyR2s openings that fail to trigger a ‘‘visible’’ Ca2+ spark. Additionally, the fraction of non-spark or ‘‘invisible’’ SR Ca2+ leak increases as SR Ca2+ content declines.

3027-Pos Board B132
GPU-Enabled stochastic Spatiotemporal Model of Rat Ventricular Myocyte Calcium Dynamics
Tuan M. Hoang-Trong, George S.B. Williams, Jonathan W. Lederer, Saleet Jafri.
The dysfunction of the normal calcium dynamics is a major factor in certain types of cardiac arrhythmias. These cardiac arrhythmias are thought to result from Ca^{2+} waves which occur when Ca^{2+} release propagates from one release site to another outside of the normal time during systole resulting in depolarization of the cell's outer membrane. Experimental results suggest that the elementary event underlying calcium release at these sites is the Ca^{2+} spark and the summation of these Ca^{2+} sparks result in the global $[Ca^{2+}]_i$ transient that causes contraction. We have developed a model of the cardiac myocyte that includes the spatial organization and microsecond level resolution of clusters of ryanodine receptor (RyR) that are Ca^{2+} release channels responsible for the generation of Ca^{2+} sparks. We use this model to explore how Ca^{2+} overload, RyR Ca^{2+} sensitivity, RyR coupling, and other factors that affect the propagation of Ca^{2+} release between release sites. We will utilize our newly developed Ultrafast Markov chain Monte Carlo method which allows the rapid simulation of a whole-cell model containing 20,000 release sites, each containing 7 L-type Ca^{2+} channels and 50 RyRs. This algorithm greatly reduces computation time by using adaptive time step approach and a compact representation of the Markov chain state space. Hence, this novel method provides a powerful tool for performing stochastic cellular simulation with realistic Ca^{2+} dynamics. Also, with the availability of the next generation graphics processing units (GPU) computing architecture - codename Fermi from NVIDIA - model solution is greatly accelerated allowing the implementation of such a detailed model for the first time.

3028-Pos Board B133
Contributions of Structural t-Tubule Heterogeneities and Membrane Ca^{2+} Flux Localization to Local Ca^{2+} Signaling in Rabbit Ventricular Myocytes
Peter M. Kekenes-Huskey, Yuhui Cheng, Johan Hake, Frank Sachse, John Bridge, J.A. McCammon, Anushka Michailova.
The micro-architecture of the transverse tubular system (t-system) and the arrangement of associated proteins are central to the function of ventricular cardiomyocytes. Recently, confocal imaging and image processing was used to characterize the geometry of the t-system in rabbit ventricular cells [1]. The average diameter of single t-tubules was estimated to be 448+-172 nm with constrictions occurring every 1.87+-1.09 um along their principal axis. Here, we used mathematical modeling to investigate how local variations in t-tubular cross-sectional area and the distribution of membrane Ca^{2+}flux regulate Ca^{2+}-entry, diffusion and buffering in rabbits [2]. The current model includes a realistic geometry of a single t-tubule, its surrounding half-sarcomeres, the spatially distributed Ca^{2+}transporting proteins along the cell membrane (L-type Ca^{2+} channel, Na^{+}/Ca^{2+} exchanger, sarcolemmal Ca^{2+} pump) as well as stationary and mobile Ca^{2+} buffers (troponin C, ATP, calmodulin, Fluo-3). A finite element software package CSMOL was used to solve the coupled reaction-diffusion PDE system describing the time-dependent concentration profiles of the above-listed species.[3]. The model was parameterized according to voltage-clamp data in rabbit ventricular myocytes with Ca^{2+} release at the sarcoplasmic reticulum disabled pharmacologically [4]. The results indicate that the constrictions and spatial arrangements of membrane Ca^{2+} proteins may cause local inhomogeneities in Ca^{2+} concentration. In addition, we examined the activation of a catalytic Ca^{2+}-binding site on Na^{+}/Ca^{2+} exchanger on local Ca^{2+} gradients in the presence or absence of fluorescent dye.
[1]Savio-Galimberti *et al.*, *Biophys J* 95:2053-2062,2008.
[2]Cheng*et al.*, *PLoS CompBiol* 2010, (in press).
[3]SmoluchowskiSolver (CSMOL), http://mccammon.ucsd.edu/smol/
[4]Sobie et al., *Biophys J: Biophys Lett*:L54-L56,2008.
Supported by NBCR (NIH grant 2 P41 RR08605), NIHGM31749, NSFMCB-0506593,MCA93S013, Center for Theoretical Biological Physics, HHMI, SDSC, W. M. Keck foundation, and the Nora Eccles Harrison Cardiovascular Research and Training Institute

3029-Pos Board B134
How Does RyR2-Mediated SR Calcium Leak Fail to Cause Sparks?
Daisuke Sato, Donald M. Bers.
Recent experiments have shown that Ca leak from the sarcoplasmic reticulum (SR) can occur via spontaneous Ca sparks, non spark ryanodine receptor (RyR) mediated leak, and RyR independent pathways (Zima *et al.BJ,* 94:104a, 2008). Opening of a single RyR is expected to increase local [Ca] in the cleft space rapidly (<< 1 ms) to levels that are expected to activate neighboring RyR in that cleft via Ca induced Ca release. Our question is how and why RyR-mediated SR Ca leak becomes mainly non-spark mediated at moderate to low SR Ca loads. To answer this question, we analyze Ca flux through RyRs using a physiologically detailed mathematical model of junctional SR Ca release in which RyR gating is regulated by intra SR and cleft [Ca] ($[Ca]_{SR}$ & $[Ca]_{Cleft}$). In this model, there are one hundred RyR channels in one Ca release unit. Each RyR opens stochastically depending on $[Ca]_{Cleft}$ and $[Ca]_{SR}$. We

find that several factors contribute to the failure of one RyR channel opening to opens adjacent RyRs as $[Ca]_{SR}$ declines: 1) the lower $[Ca]_{SR}$ reduces driving force and thus limits local $[Ca]_{Cleft}$ (both absolute level and rate of rise), 2) low $[Ca]_{SR}$ can inhibit RyR open time (τ_O) which further reduces local $[Ca]_{Cleft}$ attained, 3) the low τ_O and fast $[Ca]_{Cleft}$ dissipation after closure shorten the opportunity for neighboring RyR activation, 4) at low $[Ca]_{SR}$ the RyR2 exhibits reduced $[Ca]_{Cleft}$ sensitivity. We conclude that all of these factors conspire to reduce the probability of Ca sparks as $[Ca]_{SR}$ declines, despite continued RyR-mediated SR Ca leak.

3030-Pos Board B135

Mitochondrial-SR Ca^{2+} Cycling Modulates Normal Automaticity of Rabbit Cardiac Sinoatrial Nodal Pacemaker Cells

Yael Yaniv, Harold A. Spurgeon, Alexey E. Lyashkov, Bruce D. Ziman, Edward G. Lakatta.

A coupled-clock system within sinoatrial node cells (SANC) confers robustness and regulates their normal automaticity: basal cAMP-mediated, protein kinase A-dependent phosphorylation of Ca^{2+} cycling-proteins enables sarcoplasmic reticulum (SR) to generate spontaneous rhythmic Local, subsarcolemmal Ca^{2+}Releases (LCRs) (''Ca^{2+} clock''). LCRs activate an inward Na^+-Ca^{2+} exchange current that accelerates the diastolic depolarization promoting the ensemble of surface membrane ion channels (''membrane clock'') to generate the next action potential (AP). Intracellular Ca^{2+} enters mitochondria through the mitochondrial uniporter and is extruded by the mitochondrial Na^+-Ca^{2+} exchanger. We hypothesized that mitochondrial Ca^{2+} cycling is coupled to SANC-clocks via its impact on the intracellular Ca^{2+} cycling.

Specific inhibition of Ca influx into and efflux from mitochondria in intact single isolated SANC was effected by Ru360 and CGP-37157, respectively. Changes in mitochondrial Ca^{2+} content (Ca_m) were indexed by selective quenching of the fluorescent Ca^{2+} probe, Indo-1 in the cytosol by Mn^{2+}. Ru360 decreased Ca_m to $80 \pm 8\%$ control and increased the spontaneous SANC AP firing rate to $111 \pm 1\%$. Conversely, CGP-37157 increased Ca_m to $119 \pm 7\%$ control and reduced the spontaneous AP firing rate to $89 \pm 2\%$. Blocking Ca influx into mitochondria increased the average LCR size (measured via confocal linescans images of fluo-4) from 4.2 ± 0.1 to $6.1 \pm 0.2\mu m$ and reduced the normal LCR period from 317 ± 5 to 274 ± 6ms. In contrast, inhibition of Ca efflux from mitochondria reduced LCR size to $3.6 \pm 0.1\mu m$ and increased LCR period to 389 ± 7ms. Changes in LCR period by specific inhibition of Ca^{2+} influx or efflux into and from the mitochondria from 274 ± 6 to 389 ± 7ms predicted (R^2=0.84) the concomitant changes in the spontaneous SANC AP cycle length from 349 ± 5 to 462 ± 6ms.

We conclude that Ca^{2+} cycling into and out of mitochondria interact with the SANC coupled-clock system to modulate normal automaticity.

3031-Pos Board B136

Stochastic Beat-To-Beat Variation in Periodicity of Local Calcium Releases Predicts Intrinsic Cycle Length Variability in Single Sinoatrial Node Cells

Oliver J. Monfredi, Larissa A. Maltseva, Mark R. Boyett, Edward G. Lakatta, Victor A. Maltsev.

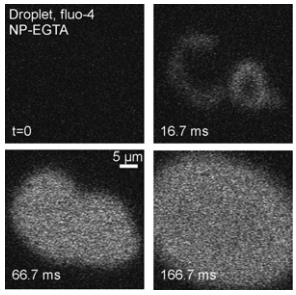
Abstract: In sinoatrial nodal cells (SANC), spontaneous, rhythmic, submembrane sarcoplasmic reticulum (SR)-generated local Ca2+ releases (LCRs) that occur during diastolic depolarization (DD) activate inward Na+/Ca2+-exchange currents that accelerate the DD rate. LCRs are roughly periodic, 'LCR period' being the time from the preceding AP-induced Ca2+-transient peak to their subsequent appearance. Previously, we demonstrated that in a given steady-state, the average LCR period of multiple AP cycles predicts concurrent average steady-state AP cycle length. We tested whether variation in LCR periods also predicts the beat-to-beat cycle length within a given steady-state. Methods: We imaged single rabbit SANC using a fast 2D-camera to capture almost all LCRs (in contrast to the relative few LCRs captured by line scan images), and in selected cells we also simultaneously measured APs by perforated patch clamp. Results: LCRs begin to occur very early during diastole, on the descending part of the prior AP-induced Ca2+ transient, shortly after the maximum diastolic potential (MDP). About 40 ms after the MDP, the ensemble of waxing LCR activity causes a late diastolic Ca elevation accompanied by a notable DD acceleration. On average, SANC (n = 9) generated 13.2 ± 3.7 LCRs per cycle, varying in size (7.1 ± 4.2μm) and duration (44.2 ± 27.1 ms). The LCR size and duration were greater for later-occurring LCRs. The average LCR period for a given cycle ranged from 70-460ms, and closely predicted (R2=0.89) the time of occurrence of the next AP, i.e. the duration of that cycle (220 to 470 ms). Numerical modeling simulations closely reproduce this experimental result. Conclusion: Intrinsic cycle length variability in single SANC is linked to cycle to cycle stochastic variations in roughly periodic LCRs.

3032-Pos Board B137

Spatially Complex Diffraction-Limited Photolysis of Caged Calcium and IP3 Combined with High-Speed Confocal Imaging

Vyacheslav M. Shkryl, Joshua T. Maxwell, **Lothar A. Blatter**.

The novel Mosaic digital illumination system (Photonic Instruments/Andor Group) integrated into a Nikon A1R confocal microscope was used to uncage Ca (DM-nitrophen, NP-EGTA) or IP_3 from multiple geometrically complex (Fig.) diffraction-limited subcellular regions and simultaneously measure $[Ca]_i$ with high-speed 2-dimensional confocal imaging (430 fps). The Mosaic System uses a computer controlled spatial light modulator to map a diffraction limited mask onto the specimen plane. A Digital Micromirror Device, consisting of a high speed array of hinge-mounted individually addressable, tiltable microscopic mirrors, directs continuous wave laser light (405 nm) onto the image plane according the user-defined diffraction limited mask. Local uncaging of Ca from multiple small regions of interest (0.63 micrometer diameter) generated artificial Ca sparks outside the cell and produced CICR inside permeabilized cardiac myocytes. Uncaging Ca from a 0.63x10 micrometer region triggered CICR and propagating Ca waves. Subsarcolemmal uncaging of IP_3 initiated propagating Ca waves that originated within the region of uncaging, and caused increased peak amplitude of electrically evoked Ca transients and Ca alternans, suggesting that in cardiac myocytes Ca release from IP_3 recpetors primes ryanodine receptor Ca release channels and enhances CICR.



3033-Pos Board B138

Calcium Spark Termination: Ryanodine Receptor Unitary Flux Dependent Mechanism

Tao Guo, Dirk Gillespie, Michael Fill.

Spontaneous sparks seem to terminate at a fixed free sarcoplasmic recticulum Ca concentration ([Ca]SR) indicating that the SR luminal Ca level is a key factor in terminating Ca sparks. In principle, such luminal Ca control could be achieved by different mechanisms. One is that luminal Ca may alter RyR2 gating by acting at intra-SR sites. Another is that, as luminal Ca falls, RyR2 unitary release flux (iCa) may become insufficient to support continued inter-RyR2 Ca-induced Ca release within a RyR2 cluster (a cytosolic process). To date, it has been virtually impossible to experimentally distinguish these possibilities in cells. We have overcome this obstacle by devising a means to manipulate iCa independently of [Ca]SR. This was accomplished by exploiting RyR2 permeation properties. Briefly, sparks and [Ca]SR were simultaneously recorded in permeabilized rat myocytes. Unitary RyR2 iCa in the tested cellular solutions was defined using single RyR2 measurements in bilayers as well as a well-established RyR permeation model. Preliminary data reveal that reducing RyR2 iCa (at a relatively constant [Ca]SR) dramatically decreases spark frequency. We believe this method is the first to experimentally delineate the contribution of RyR2 iCa flux in the SR luminal Ca control of sparks in cardiomyocytes.

3034-Pos Board B139

4-D Scanning of Calcium Sparks in Cardiomyocytes Reveals their In-Focus Amplitude

Vyacheslav M. Shkryl, Lothar A. Blatter, Eduardo Ríos.

Ca sparks, puffs and related discrete events of intracellular Ca release have so far been studied by imaging fluorescence of a suitable monitoring dye over one or two spatial coordinates and time (xt or xyt modes). By missing the vertical (z) coordinate either technique is susceptible to the out-of-focus error. This error corrupts every measurement, especially altering the measurement of amplitude, which is crucial for evaluation of numbers of contributing channels and other features with mechanistic implications. Existing theory of spark scanning (Izu et al.1998; Ríos et al. 2001) allow correction of the out-of-focus error, but only in a statistical sense, leading to correct distributions rather than accurate amplitudes of individual sparks. We now use a fast confocal slit scanner (5-LIVE; Zeiss) to image sparks in x, y and z as they evolve in time, and take advantage of the added dimension to characterize those sparks that are in focus. The distribution of amplitudes of 1196 such sparks (33 cat atrial cells) was not a sum of decaying exponentials, indicating that their Ca sources are non-Markovian channels (in agreement with Ríos et al. 2001, Wang et al. 2002), due for instance to interactions within the cluster. The directly determined distribution of amplitudes was similar to that obtained by correction, according to the theory of spark scanning, of the distribution in a larger sample imaged in xt linescans (xt-to-xyzt correction). The theory is generalized to